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Keywords (max.5): microRNA, spermatozoa, fertility

Title (25): MicroRNA expression profile in spermatozoa from fertile donors

Study question (50): Which microRNAs (miRNAs) are present in spermatozoa from fertile individuals? What is the normal miRNA expression profile?

Summary answer (50): 227 miRNAs were present in all sperm samples analyzed. Among them, miR-1274b, miR-720 and miR-34b were the most abundant. The full miR-30 family and most of the let-7 members were also present. Alternatively, 460 miRNAs were only detected in some fertile donors and 67 did not appear in any case.

What is known already (75): The transcriptome of the human spermatozoa contains a complex population of RNAs including miRNAs. MiRNAs have an important role in many biological processes such us cell cycle regulation, embryo development and gametogenesis. Several authors have recently identified altered expression profiles of miRNAs in cases of male idiopathic infertility. Nevertheless, to the best of our knowledge the normal miRNAs profile in spermatozoa from fertile donors has not been described.

Study design, size, duration (50): The expression level of 754 miRNAs was evaluated in 10 fertile donors (normal karyotype, proven fertility, and normal seminal parameters) using TaqMan® Low Density Arrays (TLDAs) (Life Technologies). The study was performed between January 2012 and December 2012.

Participants/materials, setting, methods (50): Ejaculated samples were obtained from ten fertile donors. Total RNA was isolated using the Trizol method. A miRNA reverse transcription (TaqMan® MicroRNA RT) and a pre-amplification (TaqMan® MicroRNA PreAmp) were performed. TLDAs were used to evaluate the expression level of 754 miRNA. Ct values were analysed using the HTqPCR R-package.

Results and the role of chance (125): From the 754 miRNAs screened, 227 were present in all the individuals analyzed. Among them, the top-3 most abundant miRNAs were mi-1274b, miR-720 and miR-34b. Other miRNAs detected with a high expression included miR-375, miR-19b, miR-200c, miR-132 and miR-30c. Moreover, the complete miR-30 family was also present in all fertile donors, as well as most of the let-7 family members. These two miRNA families are involved in osteoblast and epithelial cell differentiation, and embryonic development processes respectively. Alternatively, 460 miRNAs were only detected in some individuals and 67 miRNAs did not appear in any sample. Only 1.3% miRNAs were non-informative as an average. To limit the role of chance, Ct values were normalized using a mean-centering restricted method.

Limitations, reasons for caution (50): The pre-designed methodology used for the miRNA profile screening was chosen for its high sensitivity, specificity, and reliability although it does not allow the discovering of new miRNAs. Nevertheless, the 754 assays included in these arrays correspond to the most well-known and characterized human miRNAs.

Wider implications of the findings (75): The normal sperm miRNAs profile can be used as a well-defined control reference for further studies in infertile populations. Later in the project, these comparisons may allow determining the contribution of miRNAs as a possible underlying cause of idiopathic male infertility and be indicative of an altered reproductive prognosis.

Study funding/competing interest(s) (30): Work supported by 2011/FE16 (Ayudas Merck-Serono), 2009/SGR00282 (Generalitat de Catalunya), and FIS/PS09-00330 (Gobierno de España). A.S-H. is recipient of 456-01-1/E2010 grant (Universitat Autònoma Barcelona). Authors declare no competing interests.

Trial registration number (20): N/A.

The abstract will need to be categorized by either one of the two following categories:

• Basic science

• Clinical science

Subsequently the abstract will need to be categorized by one of the following topics:

• Andrology / (incl. male (in)fertility, spermatogenesis, diagnostic tests, treatment, MESA, TESA, TESE, sperm donation, environmental factors related to male fertility, immunology)

• Cross border reproductive care

• Developing countries and infertility

• Early pregnancy (incl. miscarriage, recurrent miscarriages, abortion, termination of pregnancy, ectopic pregnancy, molar pregnancy)

• Embryology (incl. IVF/ICSI, gamete and embryo selection, culture, cryopreservation, vitrification, developmental biology)

• Endometriosis, endometrium, implantation and fallopian tube

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• Quality and safety of ART therapies (incl. guidelines, accreditation, EUTCD, certification, complications: premature labour, malformations, neonatal risks, multiple pregnancy, long term follow-up of children)

• Reproductive (epi)genetics (incl. (epi)genetic causes of infertility, PGD, PGS, prenatal diagnosis)

• Reproductive endocrinology (incl. ovarian reserve testing, ovarian stimulation, IVM, POF, PCOS, infancy, disorders of sexual development, puberty, adolescence, menopause)

• Reproductive epidemiology and health economy

• Reproductive surgery (female and male)

• Stem cells

• Translational research (incl. new ideas, hypotheses, new thinking, immediate applicability in practice)